MEDICAL GENETICS AND PUBLIC HEALTH

The Twenty First Hermann M. Biggs Memorial Lecture

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THE field of public health has always been noted for its ability to put to practical use the basic discoveries of the various sciences which have had something to contribute to public welfare. With medicine as a core, public health has enlisted the aid and support of bacteriology, sanitary engineering, statistics, entomology, veterinary medicine, sociology and other pertinent disciplines. Following the early use of primitive measures of sanitation, the discovery of microbes opened a new and important phase of public health, leading to the control of many infectious diseases and the inspection and supervision of food and water supplies. More recently the activities in this field have widened to include such things as city planning, zoning, building of cheap transportation and recreational areas, provision of labor-saving devices and safety measures, and the general improvement of economic conditions.

These things, which have done so much for human progress, have been almost entirely aimed at the control of unfavorable conditions of the environment. Today most of these conditions are well under control, or at least could be if enough people wanted them controlled. There remain, however, a number of stumbling blocks to human health, the basic cause of which is genetic. As the infectious and nutritional diseases are conquered one by one, the genetic anomalies and diatheses become of greater relative importance. And just as the medical bacteriologist pointed the way to the control of infectious diseases, and the biochemist to the regulation of nutritional disorders, so the medical geneticist now holds out hope for the understanding, and thus the eventual control, of the genetic dyscrasias.

From the basic study of the principles of heredity there is emerging a new science, medical genetics, which applies these principles specifi-

cally to man. From careful research in the laboratory, in hospitals, and in homes and communities, a large amount of exact information has been gathered concerning the part that genetic variability plays in the production of human traits. Just as public health measures have depended for their success not only upon the training of specialists in the field, but also upon the education of the public, so also the newest handmaiden of public health, medical genetics, is developing both specialized and public interest. So important has our knowledge of human heredity become that courses in medical genetics are becoming standard units of the medical curriculum. Popular interest has dictated the appearance of books and magazine articles on human inheritance, and public lectures such as this one are being presented under a wide variety of auspices.

The precision of our knowledge has now reached the point where definite practical applications are available and in use. These include first, prevention, that is, the instituting of preventive measures against certain diseases and abnormalities, on the basis of specific genetic backgrounds; second, diagnosis, on the basis of genetic data, of conditions difficult to identify by other means; third, genetic prognosis, that is, the furnishing of genetic advice in prospective marriages and prospective families; and fourth, the determination of non-paternity and other medico-legal and medical problems, on the basis of the various blood agglutinogens.

Let us first consider prevention. Of all the applications of medical genetics, the one potentially most valuable is in the field of preventive medicine. It is becoming more and more feasible to prevent, in the relatives of persons with genetic conditons, the appearance of the abnormality or disease. The procedure is to examine the relatives of an affected patient by means of suitable laboratory tests, in search of the early pre-clinical or laboratory signs of the trait. When these are discovered, preventive measures are instituted.

Let me cite some examples from my own experience. At the Ohio State University I work in close association with the men in the departments of medicine and surgery. One of the most brilliant and certain applications of genetic facts to prevention has received much of its impetus from my colleagues in these departments, Doctors Doan and Curtis. I refer to the prophylactic removal of the spleen in congenital hemolytic icterus. In this disease the clinical manifestations are jaundice and marked anemia, sometimes developing as an acute fatal hemoclastic

crisis if permitted to remain untreated. The condition is the result of a dominant hereditary factor, but the clinical symptoms are not always expressed in those who inherit the factor. One or another of the laboratory stigmata is, however, always present if appropriate laboratory examinations are made. These signs include a relatively high reticulocytosis for the degree of anemia, microspherocytosis (rarely macrocytosis), increased fragility in hypotonic solutions of sodium chloride, increased icterus index, and enlarged spleen.

Where one or a combination of these signs is found in a relative of a patient with clinical activity, the spleen should be removed in the potential patient as a prophylactic measure. When it is remembered that such ordinarily minor events as an infection or a broken bone, or physiologic pregnancy in the female, may precipitate a hemoclastic crisis capable of resulting in death, the value and even the urgency of preventive splenectomy are apparent.

In connection with my University duties I teach a course in medical genetics to the medical students. It is my custom at the appropriate point in the course to have presented to the class a family in which hemolytic icterus exists. Recently I called Dr. Doan and asked if he could provide a case on a designated date. He assured me that there would undoubtedly be a case in the hospital at that time.

About a year previously, a man had been operated on for hemolytic icterus, and as is our custom, his children had been called in and examined. Two of his four sons showed the early laboratory signs of the inherited trait, and were advised to have their spleens removed as a prophylactic measure. One agreed, and his spleen was successfully removed; the other refused.

The day before my class period, Dr. Doan called and said that a boy with severe hemolytic icterus in hemoclastic crisis was being sent into the hospital, and would probably serve for presentation the next day. When the hour for the class arrived, however, Dr. Doan appeared without the patient. It had been the boy who a year earlier had refused preventive splenectomy. He arrived in extremis and lived just long enough to say, "I guess I waited too long to have my spleen removed." And he had. He could have been saved by elective prophylactic surgery on purely genetic grounds.

Another example of the genetic application of preventive measures is furnished by an anomaly known as xanthoma tuberosum. It is a stor-

age disease, a lipoidosis. Like hemolytic icterus, it has both clinical symptoms and pre-clinical laboratory signs. The visible symptoms are characteristic nodules and tumors of the tendons and joints. The laboratory signs consist of increases in the blood cholesterol and cholesterol esters. The increases may be as much as to reach 1500 mg. per cent., as compared with a normal blood cholesterol of 150-300 mg. per cent. The condition may progress to cardiovascular involvement and sudden death.

Since the hypercholesteremia is transmitted on the basis of a dominant hereditary factor, and since this may be detected by appropriate laboratory tests long before the appearance of visible tumors or nodules, it may profitably be searched for in the relatives of patients with clinical manifestations. Where found, preventive dietary and other appropriate measures may be instituted, thus helping to preclude heart involvement and premature death.

These are just two examples of many that could be cited. It might be argued by the uninitiated that such preventive measures could be invoked without any knowledge of genetics, and so they could, were it possible to examine everyone in the world for every potential disease and anomaly that he might have. Obviously this is not feasible, so that it becomes imperative to do the feasible thing, which is to search for laboratory and pre-clinical stigmata in the places where they are most likely to be found, namely, in the relatives of those patients who have clinically manifested genetic conditions.

Instances like those described, involving dominant genes, provide the most striking opportunities for the application of preventive measures on genetic grounds, since the incidence of affected relatives in cases of dominant heredity is high. A dominant gene is one which produces its effect whether it is present upon both or upon only one of the chromosomes of the pair concerned. A recessive gene, on the other hand, is one which must be present upon both chromosomes of the pair in order to produce its effect. Genes occur in alternative pairs, the two members of a pair being called *alleles*. Obviously if one allele of the pair is dominant, the other must be recessive. Dominant genes are usually symbolized by capital letters, their recessive alleles by the corresponding lower case letters. Thus we may represent the dominant gene resulting in hemolytic icterus by *I*, and its recessive allele for normal activity of the spleen and hematopoietic system by *i*. Then *II* and *Ii* will be the

genotypes (genetic compositions) of individuals with hemolytic icterus, and *ii* will represent the genotype of unaffected individuals. Persons of the genotype *II* are said to be *homozygous* for the gene *I*, while those of the formula *Ii* are called *heterozygous*. These two types of individuals can not be told apart by inspection, but may sometimes be distinguished by the types of offspring they produce.

Since hemolytic icterus is relatively rare, affected persons will usually be the offspring of one affected parent and one unaffected parent, and thus heterozygous *Ii*. Such persons will transmit the gene *I* to half their offspring, who, because of the dominance of *I*, will be potential sufferers. Similarly half the brothers and sisters of an affected patient will be expected to have the gene, and thus one or another of the stigmata.

Because hemolytic icterus is rare, we have not actually seen the homozygous condition, and can only assume that it would be the same as the heterozygous condition. Such a situation is the usual one. If a mating between two persons with hemolytic icterus should occur, we would have the possibility of observing an offspring with a double dose of the gene, one gene for icterus being inherited from each parent. Such a situation might conceivably result in a more severe form of the disease, or even in a lethal form. In our laboratory we have recently showed that multiple telangiectasia, which was long thought to be due to a simple dominant gene, is actually so severe as to be lethal when it occurs in the homozygous form. Similar situations obtain in one form of brachydactyly, in Pelger's anomaly, in sebaceous cysts and possibly in spina bifida.

The fact that not all persons who inherit the gene *I* show marked clinical symptoms of hemolytic icterus is an example of one of the basic physiological principles of genes, a principle known as *expressivity*. Some genes have constant expressivity, regularly producing the same degree of manifestation of the character. Other genes have variable expressivity, resulting in different manifestations of the trait from person to person.

Through researches subsidized by the National Research Council Committee on Human Heredity, of which I am chairman, Lennox and his coworkers[1] have shown that the dominant gene for epilepsy has variable expressivity. The basic expression of the gene is an abnormal brain wave as recorded by the electroencephalograph. This abnormal recording is known as *cerebral dysrhythmia*. It is the result of a dominant gene, and may be present with no clinical symptoms, or it may

develop into clinical epilepsy. Only about one person in twenty who inherits the gene and thus has cerebral dysrhythmia will actually develop clinical manifestations.

It is obviously important to learn the underlying causes of variable expressivity, so as to limit the expressions of genes as far as possible to innocuous effects. In some cases the variability is due to epistatic and other supplementary genes, in other cases to environmental causes such as trauma and irritation. There is unlimited opportunity for research in this direction.

Other examples of variable expressivity occur in pernicious anemia, where the achlorhydria may long precede the clinical symptoms; in allergy, where the expression may run the gamut of "hay fever," asthma, urticarial rash, eczema or angioneurotic edema; in neurofibromatosis, where the gene may result in tumors, café-au-lait spots, hemorrhage into a pachydermatococele, fibroma molluscum, plexiform neuroma, or elephantiasis neuromatosa; and many others. Knowledge of the variable expressions of genes is of value in setting up preventive and prophylactic measures, and may be of considerable importance in the next practical application to be discussed, that of diagnosis.

It is becoming more and more apparent that the alert physician will find occasional opportunities to make an otherwise difficult diagnosis by taking into account the family history of the patient. Let me again cite specific examples. My colleague, Dr. Macklin,[2] has recorded a number of instructive instances. In one case a man was admitted to the hospital, having lost considerable blood by hematemesis. The diagnosis appeared to lie between gastric ulcer, cirrhosis of the liver with esophageal varices, and Banti's disease. The appropriate laboratory tests were undertaken, but it happened that the next day one of the attending physicians recalled that the man's father had telangiectasia of a mild type, with typical spider-webbing of the capillaries of the nasal mucous membrane. Since telangiectasia is inherited on the basis of a dominant factor, it occurred to the doctor that perhaps all the patient had was a telangiectatic spot in the gastric mucosa. He explored, found a large telangiectatic focus, and excised it. The symptoms promptly disappeared, and there was no later recurrence of hemorrhage. Employment of available genetic data led to correct diagnosis and hence to proper therapy.

Again, a man had a corn on his foot which had ulcerated. Various

physicians whom he consulted were unable to give him any relief. One evening he made a social call upon a distinguished neurologist who happened to be a personal friend, and in the course of the evening hesitatingly mentioned this corn which would not get better, apologizing for bringing such a small matter to the attention of a specialist. To the man's amusement the neurologist asked for an X-ray of his lumbar spine. He did this because he had formerly treated the man's two brothers for marked trophic lesions which were the result of spina bifida. Sure enough, in this patient an occult spina bifida was found, and was shown to be the cause of the ulceration.

Not only may diagnoses sometimes be made more readily through the use of genetic backgrounds, but they may often be made earlier than would otherwise be possible. A man 54 years of age was operated on for well developed gastric carcinoma on the lesser curvature, one inch from the pylorus. His 52 year old brother, though having no symptoms, was disturbed by the presence of cancer in the family. The physician suggested that the appropriate tests be run on the brother. They were made, and an identical gastric cancer was found to be developing, one inch from the pylorus. Soon the brothers were in adjoining hospital beds, but the operation on the second brother was performed much earlier than would have been otherwise possible.

The third practical application of medical genetics to public health is our increasing ability to give accurate genetic advice to families. As a result of college courses in genetics, and of popular books and magazine articles on heredity, the well-read layman is rapidly learning that it is possible to get information about the possibility of the appearance in his children of a trait that has previously occurred in the family. Parents and prospective parents are rightfully concerned about these things. Sometimes the question concerns a wanted character such as blonde hair or musical ability. More often it involves an unwanted trait such as club foot, achondroplasia, or mental deficiency.

Before attempting to answer such a question the physician must ascertain several important facts. He must first accurately identify the trait, since minor variations may represent different genetic factors involved. The ease of identification varies: club foot is readily recognized, while border-line mental deficiency may be difficult of analysis.

When the trait is accurately identified or diagnosed, the literature on human and medical genetics must be searched for recorded instances of

the hereditary mechanism involved. Particular care must be taken to note what variations in the expression of the trait have been recorded within families, what the ages of onset have been, and with what regularity the character may have appeared within families.

It is then necessary to chart the family history of the person seeking advice, and to compare his pedigree with those recorded in the literature or in the files of the physician himself, noting similarities and differences in symptoms, age of onset, and type of transmission.

Finally the possible relations of the anomaly or disease to the environment must be evaluated, and the probable environment in which the trait will develop if it appears in this family must be specified.

With all the above facts in hand it is frequently possible to make a reasonable prognosis. Let me again draw some instances from personal experience.

Within the past year I have been consulted by two families on the question of peroneal atrophy. In one case the father suffered from the disability and wished to know the possibility of its appearance in his children. He had noticed beginning lameness in his late twenties, and at forty he showed pes cavus and atrophy of the hands. He was an only child, and he remembered his mother as being moderately crippled. The late onset and moderate involvement are characteristic of the dominant form, and it was thus possible to predict that about half his children of both sexes would show the trait.

In the other instance the parents were unaffected, but their oldest child, a boy of twelve, was severely crippled. He had begun to have trouble walking at about five years of age, and the atrophy had developed rapidly. Their other children were two girls, aged seven and two, respectively. The parents wanted to know the chances of the two girls developing the atrophy, and whether or not any of these children could transmit the trait.

The onset during the first decade and the rapid crippling are characteristic of the recessive form of peroneal atrophy. In the case of recessive traits, it will be recalled, the gene must be present on both chromosomes of the pair in order that the trait be expressed. This means that it must have been inherited from both parents. In this case, since both parents were unaffected, they must have been heterozygous. In rare recessive traits, it will be unusual to find both parents heterozygous by chance. The situation is much more likely to occur, however, when the

parents are related, since, if one is heterozygous for a trait, the other, being a relative, is likely to carry the same gene. In the family under discussion, inquiry revealed that the parents were first cousins.

It was thus possible to tell the parents that any child of theirs has one chance in four of becoming crippled. Moreover, the normal children have two chances in three of carrying the gene. Even if one of them carries the gene in heterozygous form, however, it will not result in affected children unless she marries a man who is likewise heterozygous.

The foregoing cases illustrate the fact that variations in onset and severity within a trait are often the result of different genic complexes. Conversely, the discovery of various genetic bases for what is apparently a single clinical entity can lead to important clinical distinctions which may even require different therapies. In devious ways is medical genetics making its importance felt in clinical medicine and public health.

Other examples of variations in onset and severity dependent upon different genes are to be found in epidermolysis bullosa and in retinitis pigmentosa.

Recently I was asked to advise a couple with three sons and two daughters. Two of the sons, aged nine and eleven, suffered from pseudohypertrophic muscular dystrophy. A brother of the mother had died of the disease at the age of fourteen. The parents were well aware of the hopeless prognosis for the two crippled boys, but wanted information on the chances of the reappearance of the trait if the normal boy and the two normal girls should marry.

The abnormality in this case is the result of a sex-linked gene, that is, a gene carried on the X-chromosome. Since the gene is recessive, it must be on both X-chromosomes of a woman to express itself, but due to the fact that the boys having this defect usually die before reproductive age, there is little or no chance for a girl to get the defective gene from her father. This type of dystrophy is therefore confined to boys. The chromosome complex of a male includes an X- and a Y- chromosome in place of two X-chromosomes in a female. The Y-chromosome carries no genes of this sex-linked type, so that if a boy inherits the gene for dystrophy on the X-chromosome from his mother, the Y-chromosome from his father will not contribute any gene to counteract or dominate over the defective gene, which can therefore express itself.

If we represent the gene for normal muscle development by *D*, and the gene for dystrophy by *d*, boys may be either of two genotypes, *DY*

(normal) and *dY* (crippled). Girls will ordinarily be one of two genotypes, *DD and Dd,* both unaffected. The genotype *dd* (dystrophic female) can obviously not occur as long as the crippled boys die before reaching reproductive age.

The defective gene is thus carried along in the population by some of the normal girls in the dystrophic families. In the family under discussion the parents were obviously *DY* (father) and *Dd* (mother). All the boys received the Y-chromosome from the father, but had an even chance of receiving *D* or *d* on the X-chromosome from the mother. The two crippled boys, of course, received *d* and were thus *dY*. The normal brother, who was fourteen years of age, obviously received *D* and was DY. It was thus possible to state definitely that he may marry with impunity, since he can not possibly transmit the gene.

The daughters, on the other hand, both received *D* on the X-chromosome from the father. Although they were only three and five years old, respectively, it is certain that they will be unaffected. However, they have an even chance that they carry the defective gene from the mother. Thus the chances that they may bear crippled sons are fifty-fifty, regardless of whom they marry.

Not long ago I was consulted by a young couple who had an infant son suffering from an affliction of the skin in which blebs and blisters appeared wherever the skin was subject to friction. The blisters occurred not only on the skin itself, but in the mouth, nose and throat. The child had no nails and practically no teeth, and was a mass of raw flesh. The diagnosis was made of epidermolysis bullosa dystrophica. The prognosis is bad. The parents were frantic, and demanded to know whether further children would show the defect.

This type of epidermolysis is known to be the result of still another kind of gene, an incompletely sex-linked gene. Such genes are located on the homologous regions of the X- and Y-chromosomes. These chromosomes are complex, and recent work by Koller[3] has shown that they consist of three portions, as follows. First, there is a region of the X-chromosome homologous with a corresponding region of the Y-chromosome, the two parts synapsing during meiosis and forming chiasmata. Second, there is a part of the X-chromosome not homologous with any part of the Y-chromosome; and third, there is a portion of the Y-chromosome not homologous with any portion of the X-chromosome. The non-homologous regions do not undergo synapsis during meiosis.

Genes are known to be located in each of these three regions, and all such genes will be in one way or another associated with sex in their inheritance. Those located on the non-homologous portion of the X-chromosome are called sex-linked, and will be inherited in the manner just described for pseudohypertrophic muscular dystrophy. Genes located on the non-homologous region of the Y-chromosome will be confined to men, and will produce their characteristics only in men. Such traits as ichthyosis hystrix gravior and keratoma dissipatum are of this nature.

Genes located on the homologous parts of the X- and Y-chromosomes are known as incompletely sex-linked genes, and their transmission is peculiar. About half the families in which the father carries the gene will contain more affected sons and unaffected daughters than would be expected in ordinary inheritance, while the other half will contain more affected daughters and unaffected sons than would be expected. The extent of the discrepancy will depend on the precise location of the gene on the chromosome. The location can be accurately determined by modern genetic methods.

The gene for epidermolysis bullosa of the recessive type is, then, incompletely sex-linked, and located about 20 units distant from the junction of the homologous and non-homologous portions of the sex chromosomes. Since the affected child in the family was a boy, this probably represents a non-crossover, and other affected children in the family will probably be boys. Specifically, another boy in this family would have two chances in five of being affected, whereas a girl would have but one chance in ten of showing the affliction.

The foregoing examples of genetic prognosis are all instances in which sufficient information on the mode of inheritance of the abnormality was available to make accurate predictions possible. Unfortunately, complete genetic data are not equally at hand in all inherent anomalies, and such explicit information can not always be given. There is crying need for more and more research along these lines.

The fourth practical application of medical genetics includes the medico-legal and other medical outcomes of our knowledge of the inheritance of the human blood agglutinogens. It is like carrying coals to Newcastle to speak of the blood groups to a New York audience. This is the place where Dr. Landsteiner, the discoverer of the blood groups, did so much of his work; where he and Dr. Levine worked out antigens

M and N; and where Dr. Wiener is even now continuing his remarkable work on the Rh factors. Nevertheless, any discussion of medical genetics and public health would be very incomplete without at least a reference to the subject.

More than twenty years ago I spoke in New York on the inheritance of the blood groups. At that time we knew of four groups, and I thought I had a good deal to say about them. Today we deal with 3200 groups, and the number will probably soon exceed 8000 by virtue of several new antigens which are even now in the process of description.

It will be recalled, then, that in the early days of this century Landsteiner showed that when the erythrocytes of one person were mixed with the serum of another person, agglutination might occur. The reaction took place only in certain mixtures of cells and sera. It was obviously an antigen-antibody reaction, the antigen being located in the erythrocytes, the antibody in the serum. Further work revealed the fact that there were really two antigens occurring in human erythrocytes. These were named A and B. As a result it was possible to distinguish four sorts of individuals: those containing antigen A in their cells (hence spoken of as belonging to group A), those having antigen B (group B), those having both antigens (group AB), and those possessing neither antigen (group O).

A reciprocal relationship exists between antigens A and B and their corresponding antibodies, since each person has either antigen A or the antibody against A; and either antigen B or the antibody against B. Thus there occur in various human bloods natural or "normal" antibodies against A and B.

The most obvious application of this discovery was to blood transfusion, since it would be unwise to transfuse red cells into a person whose plasma would immediately clump the cells. Before any transfusion of whole blood or erythrocytes, the blood groups of donor and recipient must be determined, so that compatible blood may be given.

We were immediately intrigued by the fact that here was a means of distinguishing four kinds of people, and in our laboratory and those of other investigators, researches were quickly undertaken to determine how the blood groups were inherited. Studies of large numbers of families have disclosed the fact that antigen A is inherited on the basis of a dominant gene. Antigen B is the result of another dominant gene, an allele of the first, and a third allele in the series results in no antigen at

all. Consequently antigen A never occurs in a child's blood unless it occurred in the blood of at least one of the parents. Similarly, antigen B never appears in a child's blood unless it was present in the blood of at least one of the parents.

Although these discoveries seemed at first only of academic interest, they proved soon to be of practical importance as well. Cases of disputed paternity, for example, may be settled from data such as these. If a child, for instance, is of group A, and the mother is of group O, then we know that the antigen A in the child must have come from the father. If the alleged father should be of group B or of group O, we can state definitely that he is not the true father of the child.

Following the first world war the intensive study of blood grouping phenomena was undertaken on a large scale. One of the outcomes of these researches was the demonstration that antigen A occurs in several detectable subtypes, now named A^1, A^2 and A^3. It is thus possible to classify people into eight different groups, namely O, A^1 A^2 A^3, B, A^1B, A^2B, and A^3B.

Another outcome of blood grouping research was the demonstration that antigens A and B exist in two different chemical forms: water-soluble and alcohol-soluble. Thus individuals of groups A, B and AB may produce water-soluble antigens, in which the antigens are found not only in the cells but in the body fluids such as the saliva, milk, tears and urine. On the other hand, they may produce the antigens in alcohol-soluble form, in which case they will be restricted to the cells.

Those persons having water-soluble antigens have been named "secretors," while those whose antigens are not soluble in water are called "non-secretors." By means of special anti-O sera, even individuals of group O can be classified into these two groups.

The "secreting" ability is the result of a dominant factor, the "non-secreting" propensity being due to its recessive allele. Thus these factors may be added to the armamentarium for medico-legal use. Also they double the number of recognizable blood groups. Since any one of the eight A-B groups may be either a secretor or a non-secretor, there are 8 x 2, or 16 groups recognizable in this way.

The discovery of antigens A and B resulted from the presence in human sera of normal antibodies against them. On the assumption that there might be in human erythrocytes other antigens for which no normal antibodies existed, Landsteiner and Levine injected human cells into

rabbits. When the resulting immune sera were exhausted with appropriate human erythrocytes, the sera still selectively agglutinated other samples of human red cells. It was apparent that the rabbits had produced a specific antibody against a human agglutinogen not previously recognized. As a matter of fact, several agglutinogens were found by this technique.

Two of the new agglutinogens were named M and N. They proved to be related in such a way that a person might have M in his cells (type M), or N (type N), or both (type MN). No one lacking both M and N has been found. Through genetic studies it has been demonstrated in our laboratory and others that these antigens, like A and B, are the result of dominant genes, and consequently never appear in a child's blood unless present in the blood of at least one of the parents.[4] Moreover antigen N, like antigen A, can be subdivided into identifiable types. We recognize N^1 and N^2, so that there are five types in the M-N series, namely M, N^1, N^2, MN^1 and MN^2. Since any one of these five could be any one of the sixteen AB groups, there are 5 x 16, or 80 different groups detectable in these two series.

In addition to lacking normal antibodies, M and N have proved to be only very weakly antigenic to human beings. They are thus unimportant in blood transfusion, even in multiple transfusions. They can be used, however, as additional criteria in cases of disputed paternity.

In the course of the injection experiments carried out by Landsteiner and Levine, still another antigen lacking normal antibodies was found. It was less satisfactory to use than antigens M and N, since potent sera were difficult to obtain and standardize, and the reactions were weak and variable. The antigen was named P, and later work has resulted in more satisfactory means of detection. For one thing, certain animals have been found to be good sources of normal anti-P serum, particularly pigs.

Genetic studies reveal that antigen P, like the others mentioned, is the result of a dominant factor. Moreover it likewise occurs in two detectable subtypes, P^1 and P^2, so that with the appropriate antisera four kinds of persons can be distinguished, namely P^1, P^2, P^1P^2 and P-.

In the combined A-B, M-N and S series there were 80 different classifications of human blood. Since the P factors are independent of the others, any one of the 80 could be any one of the 4 types in the P series, thus making altogeher 80 x 4, or 320 different groups.

Continued attempts at discovering more antigens by injection of

human blood into animals gave disappointing results. Landsteiner and Wiener therefore approached the problem from a different angle. They injected blood from the monkey, *Macacus rhesus*, into rabbits. The immunized rabbit serum was found to agglutinate human cells selectively, thus identifying still another human agglutinogen which proved to be independent of all those previously studied. Using the first two letters of rhesus, the antigen was named Rh. Eighty-five per cent of white Americans proved to have the new antigen, and these persons are spoken of as Rh+. The other fifteen per cent, lacking the antigen, are called Rh–.

Genetic studies of the new antigen revealed the fact that it, too, is inherited on the basis of a dominant gene, and may therefore be used with the other antigens in medico-legal applications.

There are apparently no normal antibodies against antigen Rh in human plasmas. In this regard the situation is similar to that in antigens M and N. There is a difference, however, in regard to antigenicity, since Rh is antigenic to man. This fact leads to several extremely important clinical applications.

In blood transfusions it is essential to transfuse only Rh– blood into an Rh– person, since repeated injections of Rh+ blood may result in immunization of the recipient with hemolytic reactions of increasing severity.[5]

Not only is the Rh agglutinogen antigenic to man, but it may pass from the circulation of an embryo through the placenta into the mother's circulation, and immunize the mother if she is Rh negative. This fact led to the discovery of the cause of erythroblastosis, which had long been known to run in families, but which had never fitted into any clear genetic picture.

Even before the discovery by Landsteiner and Wiener of the Rh antigen, Levine and Stetson[6] postulated such a factor capable of immunizing a mother. They found an atypical agglutinin in the blood of the mother of a macerated fetus. When the Rh factor was announced the following year, it proved to be the immunizing agent postulated by Levine and Stetson. In 1941 Levine, Katzin and Burnham[7] demonstrated that the Rh antigen is indeed the primary cause of erythroblastosis, accounting for more than 90 per cent of the cases. Recent work of Wiener and his coworkers has established the fact that the other 10 per cent are also due to immunization reactions, either the result of the production

of univalent Rh antibodies instead of the usual bivalent agglutinins, or in occasional cases the result of antigens A and B.

In nearly all cases of erythroblastosis the mother is Rh–, the fetus Rh+. The antigen in the fetus was, of course, inherited from the father, and some of it has passed through the placenta into the circulation of the mother, immunizing the mother. The maternal immune antibody, which can be demonstrated in the mothers of erythroblastotic infants, has then passed back into the fetus, damaging the erythrocytes. The result is hemolytic jaundice or fetal hydrops or macerated fetus, the well recognized symptoms of erythroblastosis. While the agglutination of red cells is a demonstrable test tube reaction, the end result in the fetal circulation is most probably hemolysis.

As a rule the first Rh+ pregnancy of an Rh– mother serves merely to set up the immunization, while a second or later Rh+ embryo stimulates the further rapid production of antibodies, and is itself affected. Occasionally, however, a woman produces antibodies so quickly and strongly that even the first Rh+ embryo may be affected. We have some evidence in our laboratory that these affected first-born show gross abnormalities, including spina bifida. Within the past year we have seen several such cases, and these will shortly be published.

Of course, if an Rh–woman has received a transfusion of Rh+ blood previous to her first pregnancy, she may already have been immunized, and the first Rh+ fetus she carries may then be affected in the usual manner. On the other hand, some women appear to produce antibodies so slowly that several Rh+ fetuses are required before one shows the effect of the immunization.

Although the original source of anti-Rh serum was immunized rabbits, and later immunized guinea-pigs, it was soon apparent that the serum from mothers of erythroblastotic infants was the best source of test antibodies. Nowadays such mothers furnish practically all the available test sera. The study of such sera has resulted in the discovery of several types of anti-Rh antibodies, making possible the identification of corresponding new Rh antigens, and the classification of still more kinds of persons.

Not long after the discovery of the original Rh agglutinin, Wiener[8] found a human immune serum which agglutinated only 70 per cent of human bloods instead of 85 per cent. The new agglutinin identified another Rh antigen, similar to the first. The original antigen is now called

TABLE I

Types	*Observed Frequency*	*Genotypes*	*Theoretical Frequency*	*Anti Rh° .85*	*Anti Rh' .70*	*Anti Rh" .32*	*(Anti Hr°) .63*	*Anti Hr' .80*	*Anti Hr" .97*
Rh°	.024	*Rh° Rh°*	.001024	+	—	—	—	+	+
		Rh° rh	.02336	+	—	—	+	+	+
Rh° '	.543	*Rh° ' Rh° '*	.187489	+	+	—	—	—	+
		Rh° ' Rh'	.011258	+	+	—	+	—	+
		Rh° ' Rh°	.027712	+	+	—	—	+	+
		Rh° ' rh	.316084	+	+	—	+	+	+
		Rh° Rh'	.000832	+	+	—	+	+	+
Rh° "	.137	*Rh° " Rh° "*	.021025	+	—	+	—	+	—
		Rh° " Rh"	.00087	+	—	+	+	+	—
		Rh° " Rh°	.00928	+	—	+	—	+	+
		Rh° " rh	.10585	+	—	+	+	+	+
		Rh° Rh"	.000192	+	—	+	+	+	+
Rh° ' "	.132	*Rh° ' Rh° "*	.12557	+	+	+	—	+	+
		Rh° ' Rh"	.002598	+	+	+	+	+	+
		Rh° " Rh'	.00377	+	+	+	+	+	+
Rh'	.005	*Rh' Rh'*	.000169	—	+	—	+	—	+
		Rh' rh	.00499	—	+	—	+	+	+
Rh"	.002	*Rh" Rh"*	.000009	—	—	+	+	+	—
		Rh" rh	.00219	—	—	+	+	+	+
Rh' "	.000	*Rh' Rh"*	.000078	—	+	+	+	+	+
Rh—	.133	*rh rh*	.133225	—	—	—	+	+	+

The genetic basis of the Rh-Hr blood types. The theoretical genotype frequencies were derived in my laboratory from the proportions of the Rh types as recorded by Wiener. It should be noted that anti Hr° has been postulated but not yet described.

Rh° (or Rh_o) and the new one is known as Rh'. Soon afterward Levine[8] described an immune serum which contained antibodies against both antigens, and agglutinated about 87 per cent of human bloods.

Still later Wiener[8] described a third kind of human immune serum which contained a new agglutinin, related to the other two, but reacting with only 32 per cent of human bloods. The new antigen indentified by this antibody has been named Rh". Individual persons may have any one of these antigens in their erythrocytes, or any combination of them, or none at all. Thus with the appropriate antisera anyone may be placed

in one of eight Rh types, as follows: Rh°, Rh′, Rh″, Rh°′, Rh°″, Rh′″, Rh°′″ and Rh-. The last type is Rh negative, the others are all Rh positive, for one or more of the Rh antigens.

Based on the blood groups in the A-B, M-N, S and P series, we have, it will be recalled, 320 types. Since any one of these 320 could be any one of the eight Rh types, we can now distinguish 8 x 320, or 2560 blood groups altogether.

Most recently it has been observed that corresponding to each Rh factor there is a reciprocally related antigen which has been named Hr.[10] Hr is related to Rh in the same way that M is to N, so that a person homozygous for any Rh gene is negative for the corresponding Hr antigen, and vice versa. Corresponding to the three antigens Rh°, Rh′ and Rh″, three Hr factors, Hr°, Hr′ and Hr″ are postulated. Hr′ and Hr″ have already been described; Hr° will doubtless soon be discovered and delineated.

The addition of the Hr antigens to the list makes it possible to break each of the eight Rh types into further subdivisions (Table I). Using anti-Hr′ serum, for example, we may now classify Rh°′ blood into positive for Rh°′ but negative for Hr′, and positive for Rh°′ and positive for Hr′. Interestingly enough, in most cases this difference will distinguish homozygous individuals from heterozygous individuals, as may be seen from the table, and is thus of importance in genetic prognosis in families in which erythroblastosis has occurred.

Antisera against Hr′ are now available, and make it possible to readily distinguish ten Rh blood types instead of eight. Going back to the 320 types classifiable on the basis of A, B, M, N, S and P, and realizing that any of the 320 could be any one of the ten Rh-Hr types, we have now 3200 different blood groups. When antisera against Hr° and Hr″ become available in quantity, it will be possible to distinguish 27 Rh-Hr types, and thus 27 x 320, or 8640 blood groups altogether.

The different genes involved in the production of these diverse antigens occur in varying frequencies from population to population, and herein lies another whole field of research. In any population some of the alleles will be fairly common, others rather rare. Nevertheless, it would be possible for me to take a drop of blood from each one of you in the audience tonight, and with the aid of the proper sera, to place each one of you in one of these thousands of blood groups. It would be surprising if any two of you were to fall into the same group. Then

five years later I could return here, gather you together, take another drop of blood from each of you, and, without knowing the source of the samples of blood, assign each sample to the proper person by referring to my previous list, except, of course, for such duplications of blood group as might exist among you.

Returning to erythroblastosis, the question arises as to how often by chance an Rh– woman will marry an Rh+ man and produce an Rh+ child who might thus be affected. This problem, like all the problems involved in the Rh types, is one of medical genetics, and can be solved only by medical genetic methods. Its solution has led to some remarkable new concepts of Rh immunization. Without going into the mathematical methods of gene-frequency analysis, let me say only that we would expect in a population such as ours that 23.8 per cent of all children born will have one or another Rh antigen which the mother does not have.

If all such cases are potentially erythroblastotic, we should expect the incidence of erythroblastosis to be 23.8 per cent. However, the frequency of clinically diagnosed cases has never approached this figure. The recorded incidence is about one in 200 births, or about one half of one per cent. Obviously the difference between 23.8 per cent and one half of one per cent is a pretty big discrepancy, and it is of importance to explain it.

First of all, it has been observed that of the three Rh antigens, only Rh° is of any great importance in producing symptoms. There have been a very few cases reported of effects due to immunization with Rh′ or Rh″, but the number is relatively insignificant. We may confine our attention, then, to Rh°. When we compute how often a child will be expected to have Rh° when the mother lacks it, we find that the answer is 8.5 per cent. This is much closer to the observed half of one per cent, but still far enough away to demand further investigation.

Next we recall that first-born children are seldom affected. In our American population about 31 per cent of children are first-born. Eliminating these from our calculations, we would expect 6 per cent of children to have Rh°, to be born of mothers lacking Rh°, and to be second- or later-born in the family. This further closes the gap between the expected and the observed incidence of symptoms due to Rh immunization, but still leaves a discrepancy.

Looking further, we see that the cases of erythroblastosis are not

distributed randomly among the Rh– mothers, but are grouped into specific families. This suggests that the Rh° antigen may permeate the placenta only in certain Rh– mothers, or that perhaps only certain Rh– women are capable of producing potent antibodies. It may be that both these things account for the fact that not as many cases of erythroblastosis are found as can potentially occur.

Another intriguing possibility suggests itself, and the exploration of this possibility has led to suggestive results. It is conceivable that in some instances where the antigen immunizes the mother, and the antibody in turn reaches the fetal circulation, that the effects on the fetus are different from those usually recognized as classical erythroblastosis.

In various laboratories, including our own, the search has been made for such manifestations.[11] The results indicate that the effects of Rh immunization are unimportant in the production of early abortions, and in the etiology of hemolytic icterus, sickle cell anemia, hydatiform mole, ectopic pregnancies, eclampsia and specific toxemia. In undifferentiated mental deficiency, however, positive results were obtained.

In 1944, Yannett and Lieberman[12] made an examination of the blood groups and types of the children in a school for the feebleminded, and of their mothers. They found too many Rh– mothers in the group, and too many Rh+ children of Rh– mothers. Analyzing the results it was found that of 109 feebleminded children, 53 were distributed among the specific types of mental deficiency such as Mongolian idiocy and spastic paraplegia. The distribution of the Rh factor was normal in these children and in their mothers. In the 56 children classified as undifferentiated mental deficiency, however, an abnormal distribution of the Rh factor appeared. There were too many Rh– mothers and too many Rh+ children from these Rh– mothers.

These results appeared to us so suggestive that we have been investigating the blood of the undifferentiated feebleminded at the Ohio Institute for the Feebleminded, and of their mothers (Snyder, Schonfeld and Offerman,[13] 1945). The results to date are as follows. Of 169 mothers of feebleminded children, 38 are Rh–, whereas only 21 or 22 would be expected. This difference is statistically highly significant. Of 171 feebleminded children, 27 are Rh+ from Rh– mothers, whereas we should expect only 14 or 15. This deviation is also highly significant when tested statistically.

It thus appears that the immune antibodies of the mother may pro-

duce effects on the brain tissue instead of the usual symptoms of erythroblastosis. The suggestion has been made that the immediate effect of red-cell destruction is anoxia, and that this lack of oxygen, if it occurs at a time when the brain of the embryo is in a critical stage of development, may very well cause permanent mental deficiency. As a tentative estimate of the incidence of this effect I suggest a half of one per cent.

Thus the gap between the calculated incidence of effects of Rh immunization and the observed incidence has been still further closed. It is not yet completely closed, however, and the search must continue for further, as yet unknown, manifestations. In our laboratory we have recently completed an analysis of a hundred cases of dementia praecox and their mothers. The distribution of the Rh factors agrees satisfactorily with expectation in these cases.

There is some evidence that incompatibility of A and B can cause mild symptoms in cases where the fetus has one of these antigens which is lacking in the mother. Mild jaundice of group A infants from group O mothers has been reported by Polayes[14] (1945). In these cases the titer of anti-A antibodies was found to be 1:700 as compared with an average of less than 1:200.

Another recent and important contribution to the rapidly developing Rh picture is the discovery by Wiener that immunization may result in either of two kinds of antibodies: bivalent, resulting in typical agglutination, or univalent, resulting, in the presence of conglutinin, in "conglutination." It is Wiener's belief that the type of manifestation of erythroblastosis depends upon which of these kinds of antibodies is produced. Since the type of antibody production may well be the result of a genetic factor, further intriguing problems are posed by this discovery.

Still another field for speculation is opened by the recent suggestion of Butts[15] that the malarial parasite may contain an Rh-like antigen which, through immunization, produces blackwater fever as a sequella of malaria in Rh negative persons.

Through these various examples of the growth and increasing importance of medical genetics I have tried to point out its place, shoulder to shoulder with the older sciences, as an ally of public health. Someone may ask, what about a program of eugenics as a part of public health measures? My answer is that this *is* eugenics. These things I have been describing to you form the essence of modern eugenics.

In the continuing struggle for the health and well being of all mankind, medical genetics stands ready to join those sciences which have already made so much progress, in the hope and belief that its contribution may likewise prove a worthy one.

REFERENCES

1. Lennox, W. G. Gains against epilepsy, *J.A.M.A.*, 1942, *120*:449.
2. Macklin, M. T. Heredity and the physician, *Scient. Monthly,* 1941, *52*:56.
3. Koller, P. C. Genetical and mechanical properties of sex chromosomes; man, *Proc. Roy. Soc. Edinburgh,* 1937, *57*, pt. 2:194.
4. Snyder, L. H. *Medical genetics.* Durham, N. C., Duke University Press, 1941.
5. Wiener, A. S. *Blood groups and transfusion.* 3. ed. Springfield, Ill., C. C. Thomas, 1943.
6. Levine, P. and Stetson, R. Unusual case of intra-group agglutination, *J.A.M.A.*, 1939, *113*:126.
7. Levine, P., Katzin, E. M. and Burnham, L. Isoimmunization in pregnancy; its possible bearing on etiology of erythroblastosis, *J.A.M.A.*, 1941, *116*:825.
8. Wiener, A. S., Unger, L. J. and Sonn, E. B. New data on the distribution of Rh blood types, *Proc. Soc. Exper. Biol. & Med.*, 1945, *58*:89.
9. Levine, P. Mechanism of immunization by Rh factor of red blood cells; standardization of anti-Rh serum, *Arch. Path.*, 1944, *37*:83.
10. Wiener, A. S. Theory and nomenclature of the Hr blood factors, *Science,* 1945, *102*:479.
11. Snyder, L. H. Rh factor in feeblemindedness and other diseases, *Minnesota Med.*, 1946, *29*:121.
12. Yannett, H. and Lieberman, R. Rh factor in etiology of mental deficiency, *Am. J. Ment. Deficiency,* 1944, *49*:133.
13. Snyder, L. H., Schonfeld, M. and Offerman, E. M. Rh factor and feeblemindedness, *J. Heredity,* 1945, *36*:9.
14. Polayes, S. H. Erythroblastosis fetalis in mothers with Rh-positive blood; report of 6 cases, with comment on isoimmunization with "A" and "B" agglutinogens, *Am. J. Dis. Child.*, 1945, *69*:99.
15. Butts, D. A. Rh factor in blackwater fever, *Am. J. Trop. Med.*, 1945, *25*:417.